15. A pharmaceutical composition comprising:
    (a) a first component consisting of an antiasthmatically effective amount of albuterol, said albuterol consisting of about 90 to 100% by weight of its R(-) isomer; and
    (b) a second component consisting of a physiologically effective amount of a drug selected from the group consisting of bronchodilators, antihistamines and analgesics.

16. A composition according to claim 15 wherein said second component is an antiasthmatically effective amount of theophylline or terbutaline.

17. A composition according to claim 15 wherein said second component is an analgesically effective amount of a drug selected from the group consisting of aspirin, acetaminophen and ibuprofen.

18. A composition according to claim 15 wherein said albuterol is greater than 99% by weight R(-)-albuterol.

Remarks

The claims have been amended to include the amount (in functional terms) of the components to be included and to clarify the proportion of albuterol that is present as its R-isomer. Support for claim 16 is found on page 5, line 14; support for claim 17 is found on page 5, line 15 to line 16. Claim 18 replaces former claim 14 and makes it properly dependent on newly introduced claim 15.

Claims 1 to 6, 8, 9, 13 and 14 were presented in the application as filed. Claims 9, 13 and 14 have been cancelled and claims 15 through 18 have been added. Claims 1 to 6, 8 and 15 to 18 are therefore presently pending in the application.

Claims 1 to 6 and 8 stand rejected under 35 U.S.C. 103 as obvious over Chemical Abstracts. Claims 1 to 5 stand further rejected under 35 U.S.C. 103 as unpatentable over Brittain et al., Hartley et al., Hawkins et al. and Buckner et al. Claims 6 and 8 stand further rejected under 35 U.S.C. 103 as unpatentable over the latter four references in view of Chemical Abstracts. These rejections are traversed, and reconsideration is requested, for the following reasons:

The thrust of applicants' invention is the treatment of asthma while reducing the side effects associated with the administration of racemic albuterol. Side effects of drugs which, like albuterol, have a predominant β_2 agonist component, can arise from four presently recognized interactions, as discussed in the declaration under 37 C.F.R. 1.132 by Dr. Gunnar Aberg submitted herewith and rephrased below:

(a) non-adrenergic effects (there is no evidence for this among the references cited in the present case);

(b) interaction of the β-agonist with α receptors; (Second generation β-agonists like albuterol are relatively free of this problem.)

(c) interaction of the primarily β_2-agonist drug with β_1 receptors; and

(d) interaction of β_2-agonists with β_2 receptors giving rise to tachyphylaxis and perhaps to sensitization and CNS effects such as excitement and hyperkinesia.

Tachyphylaxis in response to albuterol has been demonstrated in airways [See Passowicz Muszynska Index Medicus Abstr. 91164287 (1991) (Attachment A); and Pauwels Index Medicus Abstr. 86051970 (1986)] (Attachment B). Sensitization has likewise been reported [See Chapman et al. Brit. J. Pharmacol. 99, 66P (1990)] (Attachment C). The mechanisms of these side effects are not clear and may not be the same.

The Brittain, Hartley, Hawkins and Buckner references all address the comparative interaction of albuterol isomers with β_1

vs β_2 receptors, a type (c) interaction according to the definition above. Three of these references show that there is perhaps some slight potency advantage to the use of pure R(-) albuterol vs. racemic albuterol (although Hartley shows a potency advantage to racemic albuterol), but none shows that there is any β-selectivity advantage to R over S or over racemic. On the contrary, Buckner concluded that the ratios of tracheal (β_2) to atrial (β_1) activities of R and S are indistinguishable. Side effects that are based on type (c) interactions arise from differences in receptor selectivity, and the person of ordinary skill would conclude from the teachings of these four references that there is no advantage of R over racemic in terms of expected amelioration of side effects. The Aberg Declaration establishes that the references by Brittain, Hartley, Hawkins and Buckner do not teach any expectation of decreased side effects from the administration of the pure R isomer as compared to the racemate.

Thus, at the time of filing of applicants' parent application (1/5/90), there were no teachings among the references cited that would motivate a person of ordinary skill to administer the pure R(-) isomer of albuterol for the treatment of asthma on the basis of its receptor selectivity.

What about potency? Even though applicants' disclosure does not relate to potency, does the art nonetheless encourage the person of ordinary skill to resolve and administer pure R albuterol on the basis of potency? Unless one pure enantiomer antagonizes the effects of the other, the theoretical advantage of a pure enantiomer is at most two-fold. A racemate, being a 50:50 mixture, simply acts like half a dose of the pure enantiomer and half a dose of filler. Because chemical resolution of racemic mixtures is never 100% efficient, a resolution will always yield less than 50% of the single isomer. Thus, unless one enantiomer antagonizes the effect of the other, there is no reason to suffer the loss of material attendant upon their resolution. For example, it has been known for years that

the activity of metoprolol as a β- blocker resides in its S isomer, but no one has ever marketed pure S-metoprolol because there has been no motivation to go to the trouble of removing the R isomer.

A potency ratio significantly greater than 2 between a single enantiomer and its racemate would be consistent with antagonism by one enantiomer and would provide motivation for resolving the racemate. No such teaching is found in any of the references. Choosing the single most optimistic experimental result from among the results of three tissues in only one of the four references, one may derive a 2.3 fold potency ratio for a single (R) isomer vs racemate. This falls in the range described above for "active isomer plus filler" and provides no motivation to undertake a separation of isomers. And these are the most encouraging data selected by hindsight reconstruction; the rest of the references, taken together, fairly suggest no clear preference of one isomer. Therefore, at the time of filing, the art did not suggest using pure R(-) albuterol either for lessened side effects or for potency enhancement. This conclusion is supported by the Declaration of Dr. Aberg. (The articles referred to by Dr. Aberg which have not been previously cited in this Application are included with the Declaration of Dr. Aberg as Exhibits 1, 2 and 3.)

Applicants disclose an unexpected diminution in side effects when the pure R isomer is administered. In support of this, applicants now cite two publications by the group of Morley and Chapman which appeared subsequent to the filing of the application: Morley, Chapman et al. Brit. J. Parmacol. 104 Suppl, 295P (1991) and Chapman et al. Trends in Pharmacol. Sci. 13 231-232 (1992). The significance of their disclosures is discussed in the Declaration by Dr. Aberg and copies are enclosed for the convenience of the Examiner as Exhibits 2 and 3. In these papers, the first of which was presented at a conference in September 1991, Morley et al. address the question of a distinction between a single enantiomer and racemic albuterol in

a type (d) interaction, thus supporting the concept of lessened side effects by the administration of pure R isomer.

The Morley and Chapman references disclose that the S(+) isomer in bronchial tissue causes a hypersensitivity to allergen. The authors conclude from their experiments that the desired bronchodilator effect (due to the R isomer) is prone to tachyphylaxis, while the undesired hypersensitivity (due to the S isomer) is less prone to tachyphylaxis. The authors state "It has long been recognized that use of sympathomimetics for asthma therapy is associated with a range of inconsistent or frankly paradoxical effects....our findings indicate that it may be prudent to remove enantiomers **that were previously thought to be biologically inert."** (Chapman et al. p. 232) Thus, the use of the pure R isomer is concluded to provide unexpected advantages. Applicants' disclosure of removing the S isomer so as to reduce side effects, and claims directed thereto, dating to at least January 1990 are novel and nonobvious -- particularly as evidenced by the subsequent Morley and Chapman publications.

For the foregoing reasons the rejections of claims 1-6 and 8 under 35 U.S.C. 103 are believed overcome. Reconsideration and withdrawal of the rejections are requested.

Claims 9, 13 and 14 which had been rejected under 35 U.S.C. 112 are now cancelled. Claim 15, which replaces claim 9, now clarifies that the pharmaceutical composition comprises from 90 to 100% of the R isomer. The Examiner had also asserted that former claims 9, 13 and 14 were too broad, absent recitation of amounts of ingredients. The claims have been amended to incorporate in functional terms the amounts of the ingredients. That such functional language is definite, allowable and common practice in the pharmaceutical art is illustrated in U.S. patents 4,975,426, claim 1, 4,923,898, claim 1 and 5,025,019, claim 1, copies of which are included for the convenience of the Examiner as attachments D, E and F, respectively. The rejections under 35 U.S.C. 112 are therefore believed overcome, and reconsideration and withdrawal is requested.

There being no further issues the application is believed in condition for allowance and such is requested.

Respectfully submitted,

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Registration No. 34,480

Lexington, MA 02173

Dated: February 10, 1993

F

3/5/4
07645287 91164287
[Effect on beta adrenergic receptors of tachyphylaxis on the sensitivity of smooth muscle in the bronchi to beta adrenergic receptor agonists in bronchial asthma]
WpLyw tachyfilaksji beta-adrenergicznych receptorow na wra.ANG.zliwosc miesni gLadkich oskrzeli na agoniste receptorow beta-adrenergicznych w dychawicy oskrzelowej.
Passowicz-Muszynska E
Katedry i Kliniki Chorob Wewnetrznych AM we WrocLawiu.
Pol Tyg Lek Jul 16-30 1990, 45 (29-31) p608-11, ISSN 0032-3756
Journal Code: PBY
Languages: POLISH Summary Languages: ENGLISH
Document type: JOURNAL ARTICLE English Abstract
JOURNAL ANNOUNCEMENT: 9106
Subfile: INDEX MEDICUS
The study involved 30 subjects: 15 healthy individuals and 15 patients with atopic bronchial asthma of the moderate degree. Salbutamol was administered to asthmatic patients in the intravenous infusion for 7 days. beta-adrenergic receptor density in the lymphocytes and FEV1 were evaluated before and after therapy. Moreover, isoprenaline test was carried out to evaluate the sensitivity of the bronchial smooth muscle to beta-agonist. The test was performed prior to and after salbutamol therapy. It was found that beta-receptor agonist statistically significantly decreases beta-adrenergic receptor density. Equivalently, bronchial smooth muscle is less sensitive to beta-agonist in the same degree as a decrease in beta-adrenergic receptor density in the peripheral blood lymphocytes.
Tags: Female; Human; Male
Descriptors: *Albuterol--Therapeutic Use--TU; *Asthma--Drug Therapy--DT; *Bronchi--Drug Effects--DE; *Muscle, Smooth--Drug Effects--DE; *Receptors, Adrenergic, Beta--Drug Effects--DE; *Tachyphylaxis--Physiology--PH; Adolescence; Adult; Asthma--Physiopathology--PP; Lymphocytes--Drug Effects --DE
CAS Registry No.: 0 (Receptors, Adrenergic, Beta); 18559-94-9 (Albuterol)

ATTACHMENT A

G

3/5/18
05750970 86051970
[Effect of corticosteroids on the action of sympathomimetics]
Influence des corticosteroides sur l'action des sympathicomimetiques.
Pauwels R
Bull Eur Physiopathol Respir Sep-Oct 1985, 21 (5) p53s-55s, ISSN 0395-3890 Journal Code: BGX
Languages: FRENCH Summary Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW English Abstract
JOURNAL ANNOUNCEMENT: 8603
Subfile: INDEX MEDICUS

Corticosteroids restore the bronchial responsiveness to beta-adrenergic stimulants in man. This has been shown both in severe asthmatic patients and in normal subjects, rendered insensitive by artificial means. On the contrary, in patients with bronchial asthma who have airways reactive to beta-adrenergic stimulants, the combination of corticosteroids and sympathicomimetics results in an additive effect of their bronchodilating capacity. Animal models, both in vivo and in vitro, show the same type of interaction between corticosteroids and beta-adrenergic stimulants. The mechanism by which corticosteroids restore the bronchial sensitivity to beta-adrenergic stimulation is not completely understood. Several mechanisms may be involved such as increased agonist binding, decreased receptor turn-over, increased uncoupling between receptor and adenylcyclase, decreased extraneuronal uptake, decreased COMT-activity. The relevance of the influence of corticosteroids on the metabolism of membrane phospholipids remains highly speculative. (15 Refs.)

Tags: Human
Descriptors: *Adrenal Cortex Hormones--Therapeutic Use--TU; *Adrenergic Beta Receptor Agonists--Therapeutic Use--TU; *Asthma--Drug Therapy--DT; Albuterol--Therapeutic Use--TU; Bronchodilator Agents--Therapeutic Use--TU; Drug Synergism; Drug Tolerance; Hydrocortisone--Therapeutic Use--TU; Isoproterenol--Therapeutic Use--TU; Methylprednisolone--Therapeutic Use--TU ; Prednisolone--Therapeutic Use--TU; Pregnenediones--Therapeutic Use--TU; Tachyphylaxis; Terbutaline--Therapeutic Use--TU
CAS Registry No.: 0 (Adrenal Cortex Hormones); 18559-94-9 (Albuterol) ; 23031-25-6 (Terbutaline); 50-23-7 (Hydrocortisone); 50-24-8 (Prednisolone); 51333-22-3 (budesonide); 7683-59-2 (Isoproterenol); 83-43-2 (Methylprednisolone)

ATTACHMENT B

65P PERTUSSIS TOXIN INDUCES BRONCHOPULMONARY HYPERREACTIVITY TO HISTAMINE, 5-HT AND ANTIGEN IN GUINEA-PIGS, BUT SUPPRESSES RESPONSES TO FMLP

A. Imaizumi*, J. Lefort & B.B. Varg[redacted]. Unité de Pharmacologie Cellulaire, Unité Associée Institut Pasteur-INSERM n° 285, 25 rue du Dr Roux, 75015, Paris, France.

Mice and rats inoculated with *Bordetella pertussis* vaccine show increased sensitivity to histamine, serotonin and anaphylaxis (Parfentjev and Goodline, 1948; Kind, 1958). This has been attributed to an acquired imbalance of two adrenergic effector systems, i.e., to a reduced functioning of the ß-adrenergic receptors or of some of the reactions between receptor activation and adrenergic end-response (Szentivanyi, 1968). We have shown that enhanced bronchoconstriction, BC (i.e., unspecific broncho-pulmonary hyperresponsiveness) follows the administration of a booster injection of antigen to actively sensitized guinea-pigs (Pretolani et al., 1988). This led us now to study the effects of pertussis toxin (PT), the active component of *B. pertussis* on broncho-pulmonary responsiveness. PT was administered i.v. to guinea-pigs at 0.8-20 µg/kg 6-72 h before they were stimulated, under pentobarbitone anesthesia, with i.v. histamine (0.5-16 µg/kg) or serotonin (0.5-8 µg/kg), at 10 min intervals. Bronchial resistance to inflation was evaluated by the method of Konzett-Rössler in cm H_2O. PT induced leukocytosis (lymphocytosis), and in 10 animals the number of circulating leukocytes increased from 5,700±800 to 38,900±3,700 at the dose of 20 µg/kg after 72 h. This effect was dose and time-dependent and started within 6 h. Initially no differences were observed between the bronchoconstrictor responses to histamine or to serotonin of control and PT-treated animals but, when propranolol was used (1 mg/kg i.v. and 3 mg/kg i.p.), BC was slightly increased only (% BC: 13.4±2.8 up to 19.6±3.5) in control, but was markedly increased (% BC: 8.9±2.8 to 70.5±4.4, $p<0.001$) in animals treated 72 h beforehand with PT at 20 µg/kg. Similar effects were observed with serotonin. In contrast, BC and the accompanying leukopenia induced by the i.v. administration of the secretagogue N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) (Boukili et al., 1986 and 1989) were antagonized by PT. Because of the contrasting effects on fMLP and on histamine and serotonin, isolated lungs provided by PT-treated animals where used . Under those conditions, BC and histamine and thromboxane A2 releases induced by the intra-pulmonary administration of fMLP were suppressed but the effects of OA (3 ng-100 µg injected to the lungs of guinea-pigs immunized with 10 µg ovalbumin (OA) in Al(OH)3 injected i.p. twice, at a 2-week interval) were enhanced. PT thus modifies negatively the signal transductions for cells involved in the lung responses to fMLP, but positively the effects of the direct constrictor agents histamine and serotonin and of antigen, which induces BC via these mediators. Our data suggest that PT prevents the effects of fMLP on a target other than the neutrophil, since it was effective on the isolated lungs (Boukili et al., 1989), possibly via its recognized effects on the Gi protein of other effector systems present in the lung. Hyperresponsiveness may result from an enhanced mediator release, possibly due to down regulation of a Gi protein, associated to a direct effect on smooth muscle, at a level which is under investigation.

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66P AN ANOMALOUS EFFECT OF SALBUTAMOL IN SENSITISED GUINEA-PIGS

I. Chapman*, L. Mazzoni & J. Morley, Preclinical Research, Sandoz Ltd., Basel CH-4002, Switzerland.

Eosinophils migrate to the intrapulmonary airways of sensitised guinea-pigs in response to inhaled allergen. Whilst assessing the capacity of anti-asthma drugs to inhibit this phenomenon, it was noted that animals pretreated with salbutamol (S) (1 mg/kg/day) by subcutaneous infusion invariably died on inhalation of allergen, in marked contrast to animals that were untreated or received other anti-asthma drugs. The contribution of altered airway smooth muscle function to this untoward effect has been investigated.

Guinea-pigs (450-600 gm) were sensitised by intraperitoneal injection (1 ml) of a suspension containing ovalbumin (OA, 10 ug/ml) and aluminium hydroxide (10 mg/ml) and separately with pertussis toxin (0.25 ml) on day 0, boosted on day 14 and implanted with either saline (C) or salbutamol (S) (1 mg/kg/day, Alzet minipump, s.c.) between day 21 and day 30. Six days later animals were anaesthetised with phenobarbitone (100 mg/kg i.p.) and pentobarbitone (30 mg/kg i.p.) paralysed with gallamine (10 mg/kg i.m.) and ventilated (1 hz, 8 ml/kg) via a tracheal cannula. Airway resistance (R_L cm H_2O/l/sec) and compliance (C_{dyn} ml H_2O/l/sec) were calculated from measurement of tracheal airflow and transpulmonary pressure (Digital electronic pulmonary monitoring system, Mumed Ltd., U.K.). Animals were challenged with aerosolised OA (10-1000 ug/ml for 10 min) and changes in R_L and C_{dyn} were monitored at each breath. Airway responses to inhaled OA or intravenous histamine (1.0 & 1.8 ug/kg) were expressed as the maximal increase in R_L (mean±sem).
Responses to histamine in naive animals (107±67, 198±77, n=4) were not dissimilar from C animals (109±48, 262±91, n=10). Prior treatment with S (1 mg/kg/day s.c.) resulted in a slight reduction of these responses (46±12, 139±42, n=10, NS). No response to inhaled OA (100 ug) was observed in naive animals, in contrast to C animals (132±38, n=10) which developed increased reactivity to histamine following antigen challenge (418±64, 799±76, n=10). In animals pretreated with S, the reaction to antigen (334±58, n=10) was significantly ($P<0.001$) increased, even though airway responses to histamine were slightly reduced (225±66, 613±106, n=10).

The present results demonstrate that pretreatment of sensitised guinea-pigs with S augments the response to antigen. Altered distribution or increased dosage of inhaled allergen, altered airway reactivity or hypoxic vasoconstriction are mechanisms that might contribute to this phenomenon.

ATTACHMENT C

United States Patent [19]
Sunshine et al.

[11] Patent Number: 4,975,426
[45] Date of Patent: Dec. 4, 1990

[54] COUGH/COLD MIXTURES COMPRISING NON-SEDATING ANTIHISTAMINE DRUGS

[75] Inventors: Abraham Sunshine, New York; Eugene M. Laska, Larchmont; Carole E. Siegel, Mamaroneck, all of N.Y.

[73] Assignee: Analgesic Associates, Larchmont, N.Y.

[21] Appl. No.: 315,161

[22] Filed: Feb. 24, 1989

Related U.S. Application Data

[62] Division of Ser. No. 59,635, Jun. 8, 1987, Pat. No. 4,829,064.

[51] Int. Cl.[5] A61K 31/60; A61K 31/62; A61K 31/615; A61K 31/505; A61K 31/44; A61K 31/445; A61K 31/19

[52] U.S. Cl. 514/159; 514/161; 514/165; 514/166; 514/256; 514/290; 514/315; 514/336; 514/570

[58] Field of Search 514/159, 165, 256, 290, 514/315, 336, 570, 629, 630

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Primary Examiner—Douglas W. Robinson
Assistant Examiner—Raymond J. Henley, III
Attorney, Agent, or Firm—Burns, Doane, Swecker & Mathis

[57] ABSTRACT

Pharmaceutical compositions and methods of using same comprising aspirin, sodium salicylate, salicylamide or acetaminophen, in combination with a non-sedating antihistamine and optionally one or more other active components selected from a decongestant, cough suppressant (antitussive) or expectorant are provided for the relief of cough, cold, cold-like and/or flu symptoms and the discomfort, pain, headache, fever and general malaise associated therewith.

33 Claims, No Drawings

ATTACHMENT D

[19] United States Patent
Sunshine et al.

[11] Patent Number: 4,923,898
[45] Date of Patent: May 8, 1990

[54] ANALGESIC, ANTI-INFLAMMATORY AND SKELETAL MUSCLE RELAXANT COMPOSITIONS COMPRISING NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AND MUSCULOSKELETAL RELAXANTS AND METHODS OF USING SAME

[75] Inventors: **Abraham Sunshine**, New York; **Eugene M. Laska**, Larchmont; **Carole E. Siegel**, Mamaroneck, all of N.Y.

[73] Assignee: **Analgesic Associates**, Larchmont, N.Y.

[21] Appl. No.: **227,989**

[22] Filed: **Aug. 3, 1988**

Related U.S. Application Data

[60] Division of Ser. No. 114,751, Oct. 30, 1987, Pat. No. 4,780,463, which is a division of Ser. No. 815,502, Jan. 2, 1986, Pat. No. 4,722,938, which is a continuation of Ser. No. 686,380, Dec. 26, 1984, abandoned.

[51] **Int. Cl.**[5] **A61K 31/19**
[52] **U.S. Cl.** **514/557**
[58] **Field of Search** 514/557

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Primary Examiner—Stanley J. Friedman
Attorney, Agent, or Firm—Burns, Doane, Swecker & Mathis

[57] **ABSTRACT**

Novel pharmaceutical analgesic, anti-inflammatory and skeletal muscle relaxant compositions and methods of using same comprising an analgesically and anti-inflammatory effective amount of at least one non-steroidal anti-inflammatory drug other than aspirin, acetaminophen and phenacetin, in combination with an effective amount of a skeletal muscle relaxant.

20 Claims, No Drawings

ATTACHMENT E

United States Patent [19]
Sunshine et al.

[11] Patent Number: 5,025,019
[45] Date of Patent: Jun. 18, 1991

[54] COUGH/COLD MIXTURES COMPRISING NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

[75] Inventors: Abraham Sunshine, New York; Eugene M. Laska, Larchmont; Carole E. Siegel, Mamaroneck, all of N.Y.

[73] Assignee: Analgesic Associates, Larchmont, N.Y.

[21] Appl. No.: 438,074

[22] Filed: Nov. 20, 1989

Related U.S. Application Data

[62] Division of Ser. No. 144,099, Jan. 15, 1988, Pat. No. 4,920,149, which is a division of Ser. No. 887,205, Jul. 21, 1986, Pat. No. 4,738,966, which is a division of Ser. No. 752,546, Jul. 8, 1985, Pat. No. 4,619,934, which is a division of Ser. No. 598,502, Apr. 9, 1984, Pat. No. 4,552,899.

[51] Int. Cl.[5] A61K 31/19; A61K 31/44; A61K 31/435; A61K 31/445

[52] U.S. Cl. 514/277; 514/290; 514/325; 514/568; 514/653

[58] Field of Search 514/568, 653, 277, 290, 514/325

Primary Examiner—Stanley J. Friedman
Attorney, Agent, or Firm—Burns, Doane, Swecker & Mathis

[57] ABSTRACT

Pharmaceutical compositions and methods of using same comprising a non-steroidal anti-inflammatory drug in combination with at least one other active component selected from an antihistamine, decongestant, cough suppressant (antitussive) or expectorant are provided for the relief of cough, cold and cold-like symptoms.

23 Claims, No Drawings

ATTACHMENT F

0701.027

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ny of Science .U.S.A.

THE β-ADRENERGIC RECEPTOR KINASE: ROLE IN HOMOLOGOUS DESENSITIZATION IN S49 LYMPHOMA CELLS

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Summary

Phosphorylation of the β-adrenergic receptor (βAR) is closely associated with homologous desensitization of the β-adrenergic receptor-coupled adenylate cyclase system. Homologous desensitization and receptor phosphorylation also occur in cell mutants which are deficient in their cAMP-dependent protein kinase (kin^- mutant of S49 lymphoma cells). βAR phosphorylation is mediated by a cAMP-independent protein kinase which phosphorylates the receptor only when it is occupied by a β-agonist. During the time course of desensitization the βAR kinase (βARK) activity is translocated from a cytoplasmic to a plasma membrane location. βARK translocation can also be effected by prostaglandin E_1 (PGE_1) suggesting that this βARK may represent a more general enzyme capable of phosphorylating other adenylate cyclase-coupled receptors. Thus, βARK may play a key role in the process of homologous desensitization of adenylate cyclase coupled receptors.

Extracellular hormones interact with specific receptors at the outer surface of the plasma membrane and thus initiate a cellular response. One of the best studied transmembrane signalling systems known to be coupled to the occupancy of cell surface receptors is adenylate cyclase. The adenylate cyclase system is composed of various components all of which have been purified to homogeneity (Shorr et al., 1982; Homcy et al., 1983; Benovic et al., 1984; Codina et al., 1984; Northup et al., 1980; Sternweis et al., 1981; Bokoch et al., 1984; Pfeuffer et al., 1985). Initially, agonist binding to the receptor promotes coupling of the occupied receptor to one of the guanine nucleotide binding regulatory proteins. These proteins are members of a

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EXHIBIT 1

family of heterotrimeric proteins consisting of α, β and γ subunits. Stimulatory receptors like the β-adrenergic (Cerione et al., 1984) or glucagon (Iyengar et al., 1979) receptors couple to the stimulatory regulatory protein N_s (or G_s) whereas inhibitory receptors like the α_2-adrenergic (Jacobs et al., 1976) or M_2-muscarinic (Harden et al., 1982) receptors couple to the inhibitory regulatory protein N_i (or G_i).

Prolonged exposure to agonist hormones, either stimulatory or inhibitory, results in an attenuation of the response to the hormonal activation, a phenomenon called tachyphylaxis or desensitization (Harden, 1983; Sibley and Lefkowitz, 1985; Sharma et al., 1975). One of the best studied models for desensitization is the β-adrenergic receptor-coupled adenylate cyclase system. In this system two different forms of desensitization have been characterized. Homologous or hormone-specific desensitization results in an attenuated response only to the desensitizing hormone. In contrast, the heterologous form of desensitization leads to a general decrease of adenylate cyclase activity promoted not only by the desensitizing hormone but by other hormones and non-hormonal stimulators as well.

Previous studies have demonstrated that phosphorylation of the β-adrenergic receptor is involved in the mechanism of heterologous desensitization (Stadel et al., 1983; Sibley et al., 1984). In this form of desensitization phosphorylation of the β-adrenergic receptor is at least in part cAMP-dependent and mediated by the cAMP-dependent protein kinase (protein kinase A) (Strulovici et al., 1984; Sibley et al., 1984; Benovic et al., 1985).

Homologous desensitization, however, appears to be independent of cAMP since it has been observed in systems which are defective in their cAMP-dependent pathway (Green and Clark, 1981; Green et al., 1981; Perkins, 1983; Clark et al., 1985). These systems either lack the N_s protein or a functional cAMP-dependent protein kinase. Consequently β-adrenergic receptor occupancy does not result in an increase in intracellular cAMP levels (cyc^- mutant of S49 lymphoma cells) (Bourne et al., 1975; Bourne et al., 1981; Ross and Gilman, 1977) or cAMP-dependent protein phosphorylation (kin^- mutant of S49 lymphoma cells) (Steer et al., 1976; Steinberg et al., 1978; Mahan et al., 1985). Therefore, if phosphorylation of the β-adrenergic receptor is involved in the process of homologous desensitization it must be catalyzed by a non cAMP-dependent protein kinase. To address these questions we utilized the kin^- mutant of the S49 lymphoma cells (Steer et al., 1976; Steinberg et al., 1978; Mahan et al., 1985). We document here a cAMP independent pathway of β-adrenergic receptor active phosphorylation during homologous desensitization. The kinase involved in this phosphorylation

process is distinct from other known kinases and phosphorylates only the agonist occupied form of the β-adrenergic receptor. Moreover, during desensitization the cytosolic kinase activity becomes transiently translocated to the plasma membranes in a cAMP-independent manner.

MATERIALS AND METHODS

Cells and incubations - S49 lymphoma cells, wild type (clone 24.3.2) and kin^- mutants (clone 25.6.1), were grown in Dulbecco's modified Eagle's medium with 10% horse serum. Cells were harvested by centrifugation (800 x g, 3 min), washed three times with phosphate-free Dulbecco's modified Eagle's medium and incubated at 37°C for various periods of time (as indicated) in the presence of a β-adrenergic agonist for desensitization. To study the *in situ* phosphorylation of the β-adrenergic receptor the intracellular pool of ATP was labeled by incubating the cell with carrier-free ^{32}P (0.3 mCi/ml) prior to desensitization. The desensitization incubation was stopped by adding ice-cold phosphate-buffered saline with propranolol (10^{-6} M) followed by immediate sedimentation of the cells (800 x g, 5 min).

Purification of the β-adrenergic receptor - The purification of the *in situ* phosphorylated β-adrenergic receptor was performed by affinity chromatography as previously described (Strasser et al., 1986a). Purified β-adrenergic receptor from hamster lung (Benovic et al., 1984) was used as a substrate for the receptor kinase assays.

Preparation of cell fractions for assay of β-adrenergic receptor kinase - After incubation (as described above) the sedimented cells were lysed in 2 volumes of 10 mM Tris, 15 mM $MgCl_2$, 5 mM EDTA, 10^{-4} M PMSF, 5 μg/ml leupeptin, 5 μg/ml pepstatin, pH 7.4 using a glass homogenizer (20 strokes). Unbroken cells and cell nuclei were sedimented at 800 x g for 10 min and discarded. The plasma membranes were then sedimented at 48,000 x g for 20 min. To obtain a cytosolic fraction the 48,000 x g supernatant was centrifuged at 150,000 x g for 60 min. To test for the receptor kinase activity the cytosolic and plasma membrane fractions were used directly.

Kinase assay - Pure β-adrenergic receptor was reconstituted into phospholipid vesicles as previously described (Benovic et al., 1986). The reconstituted β-adrenergic receptor (≅ 5 pmol) was incubated in 25 mM Tris, 10 mM NaCl, 1.5 mM EDTA, 1 mM EGTA, 5 mM $MgCl_2$, 5 mM NaF, 50 μM Na_3VO_4, 10^{-4} M PMSF, 5 μg/ml leupeptin, 5 μg/ml pepstatin, pH 7.4 in the presence of 50 μM [γ-^{32}P]ATP (25 cpm/fmol), with or without 10^{-4} M isoproterenol or the β antagonist alprenolol (10^{-5} M) and in the presence of the appropriate kinase preparation for 20 min at 30°C in a

total volume of 100 µl. The reaction was stopped by adding 1 ml of ice-cold 100 mM NaCl, 10 mM Tris, 2% digitonin, pH 7.2. The β-adrenergic receptor was then repurified by affinity chromatography (Benovic et al. 1986).

Other assays - β-Adrenergic receptor assays, adenylate cyclase assays and $NaDodSO_4$/polyacrylamide gel electrophoresis were performed essentially as described in Strasser et al. (1986a).

RESULTS

Wild type (WT) and kin^- mutants of the S49 lymphoma cells preincubated with carrier-free [^{32}P]Pi to label the intracellular ATP pool (Strasser et al., 1986a), were incubated in the presence of 10^{-4} M isoproterenol to induce desensitization. Homologous desensitization (agonist specific) was documented by measuring the adenylate cyclase activity in the plasma membranes (data not shown). As shown in Fig. 1 homologous desensitization induces a dramatic increase in the phosphorylation of the β-adrenergic receptor of both the wild type and the kin^- mutant of the S49 lymphoma cells (0.2 mol P/mol β-adrenergic receptor for control and 0.8 mol P/mol for desensitized cells). These results indicate that a non cAMP-dependent pathway is involved in the phosphorylation process of the β-adrenergic receptor during homologous desensitization.

To identify the kinase activity which is involved in this phosphorylation process, the cytoplasmic and plasma membrane fractions from untreated kin^- mutants of the S49 lymphoma cells were tested for their ability to phosphorylate pure βAR reconstituted into phospholipid vesicles. As shown in Fig. 2 cytoplasmic fractions of these cells phosphorylate the βAR but only in the presence of the β-agonist isoproterenol. The presence of the β-agonist induces about a 5-to 10-fold increase in the phosphorylation of the βAR. The effect of the agonist can be completely blocked by the β antagonist alprenolol. These data indicate that in the reconstituted system agonist occupancy of the βAR induces a state of the receptor which makes it a much better substrate for βARK activity present in the cytosol of these cells. This effect of agonist is independent of the generation of cAMP or presumably any other unknown second messenger since the effect is observed in an *in vitro* system utilizing purified components.

As mentioned above the β-adrenergic receptor kinase is a predominantly cytosolic enzyme. Yet the β-adrenergic receptor is an integral membrane glycoprotein (Stiles et al., 1984). Thus, the question arises as to how does a cytosolic enzyme function to

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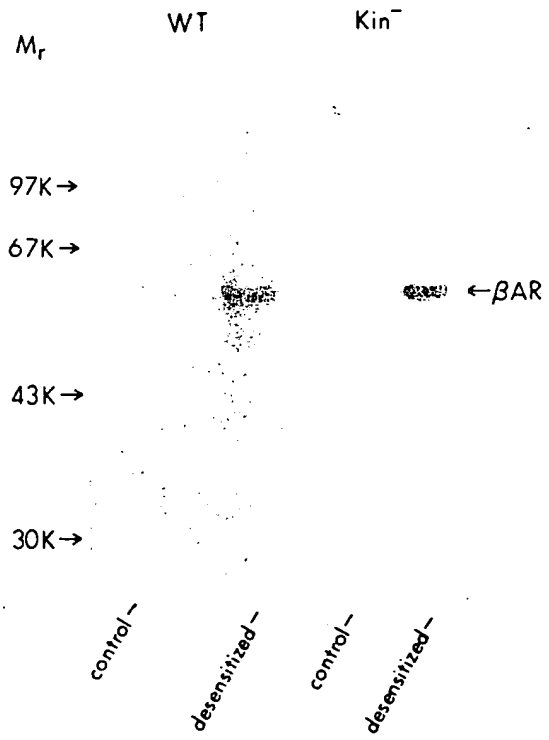

Fig. 1. Phosphorylation of the β-adrenergic receptor during desensitization in WT and kin⁻ S49 lymphoma cells. Wild type and kin⁻ mutants of the S49 lymphoma cells were incubated (37°C) with 0.3 mCi $^{32}P_i$/ml Pi as described in Methods. Desensitization was induced by incubating the cells with isoproterenol (10^{-5} M) for 20 min. The β-adrenergic receptors were purified and visualized by autoradiography after gel electrophoresis (see Methods). Indicated on the left is the relative mobility of the molecular weight standards. Indicated on the right (arrow) is the relative mobility of the β-adrenergic receptors derived either from control (lane 1) or desensitized (lane 2) wild type cells or control (lane 3) or desensitized (lane 4) kin⁻ mutant cells.

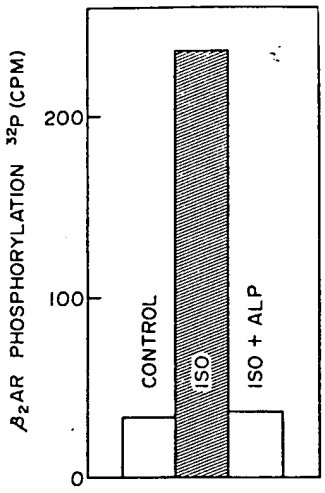

Fig. 2. Influence of agonist occupancy on phosphorylation of the β-adrenergic receptor by the β-adrenergic receptor kinase. Pure hamster lung β-adrenergic receptor was reconstituted into lipid vesicles and incubated for 30 min at 30°C with crude β-adrenergic receptor kinase prepared from a kin$^-$ cell cytosol fraction. The incubations also contained either no ligand (control), 100 μM (-)isoproterenol (Iso) or 100 μM (-)isoproterenol + 10 μM (±)alprenolol (Iso + Alp). Phosphorylated β-adrenergic receptor was then repurified, electrophoresed on a 10% polyacrylamide gel and visualized by autoradiography (see Methods).

phosphorylate a plasma membrane protein? In an attempt to answer this question we followed cytoplasmic enzyme activity and in situ phosphorylation of the β-adrenergic receptor as a function of time of exposure to isoproterenol. As the β-adrenergic receptors become rapidly phosphorylated, the β-adrenergic receptor kinase activity rapidly disappears from the cytosolic fraction (Fig. 3). After 15 min of

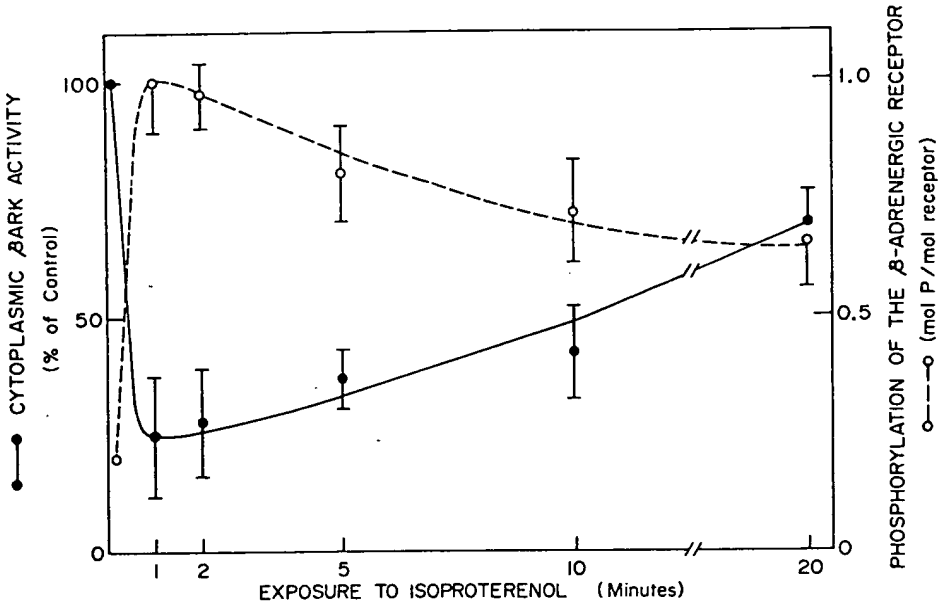

Fig. 3. Time course of cytoplasmic βARK activity and in situ β-adrenergic receptor phosphorylation during desensitization. Kin$^-$ mutants of the S49 lymphoma cells were incubated 0-30 min in the presence of 10^{-5} M isoproterenol to induce homologous desensitization. The β-adrenergic receptor kinase activity relative to control (●) was measured using the reconstituted, agonist occupied hamster lung receptor as substrate (see Methods). The phosphorylation of the β-adrenergic receptor (○) within the plasma membrane of the intact cells (in situ) was quantitated after autoradiography of the purified receptor (see Methods).

isoproterenol induced desensitization about 75% of the kinase activity has vanished from the cytosol (Fig. 3). This decrease in cytosolic kinase activity is accompanied by a simultaneous increase in the kinase activity associated with the plasma membrane. As shown in Fig. 4, an increase in membrane activity of about 6.5 fold can be observed indicating that the β-adrenergic receptor kinase is translocated from the cytosol to the plasma membrane upon β agonist promoted desensitization. At longer times (Fig. 3) (20-60 min) when the extent of phosphorylation of the total pool of receptor decreases the cytosolic kinase activity returns to control levels (data not shown).

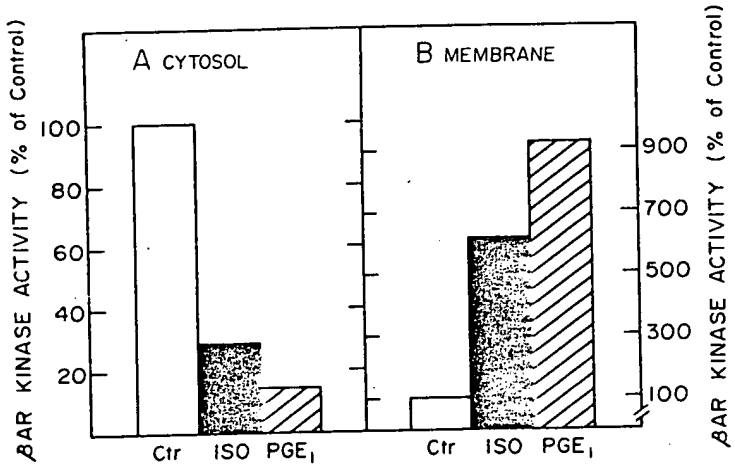

Fig. 4. Translocation of the β-adrenergic receptor kinase from the cytosol to the plasma membrane. Kin^- mutants of the S49 lymphoma cells were desensitized for 15 min with 10^{-5} M isoproterenol (ISO) or 10^{-6} M prostaglandin E_1. The β-adrenergic receptor kinase activity was measured in the cytoplasmic (cytosol) and in the plasma membrane (membrane) fractions using the reconstituted, agonist occupied β-adrenergic receptor as substrate (see Methods). Indicated are the relative kinase activities compared to controls.

These data suggests that specific agonist occupancy of the β-adrenergic receptor triggers the translocation of the receptor kinase. We next wished to determine whether this kinase is a specific β-receptor kinase or whether it is an enzyme with more general substrate specificity. Since the β-adrenergic receptor is the only adenylate

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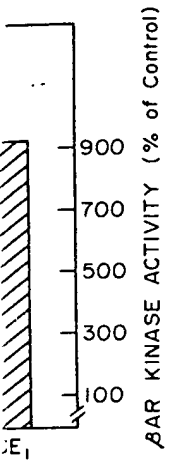

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cyclase stimulatory receptor purified to homogeneity we attempted to use this translocation phenomenon of the kinase to further probe the specificity of this kinase. S49 lymphoma cells are known to possess prostaglandin E_1 (PGE_1) receptors coupled to stimulation of adenylate cyclase (Bourne et al., 1982). As has been shown previously (Strasser et al., 1986) prolonged exposure of S49 lymphoma cells to PGE_1 induces a homologous form of desensitization to PGE_1 stimulation of adenylate cyclase. Strikingly, PGE_1 induced desensitization of the PGE_1 stimulated adenylate cyclase also promotes a translocation of the receptor kinase activity from the cytosol to the plasma membrane (Figure 4).

DISCUSSION

The data presented here document that: 1) β-adrenergic agonists can stimulate the phosphorylation of their own receptors, the β-adrenergic receptor, via a cAMP-independent pathway. 2) This phosphorylation is carried out by a kinase (βARK) which is exquisitely specific for the agonist occupied form of the β-adrenergic receptor. 3) βARK is a cytosolic enzyme which appears to translocate to the plasma membrane upon occupancy of the β-receptor with an agonist. 4) βARK may have a broader specificity since other stimulators of adenylate cyclase such as PGE_1 will promote the translocation of the activity from cytosol to plasma membrane. 5) Phosphorylation of the β-adrenergic receptor by βARK appears to correlate temporally with the process of homologous desnsitization in S49 cells.

Moreover, this receptor kinase activity has been separated from other known kinase activities by sequential chromatography on molecular sieve HPLC and DEAE chromatography (Benovic et al., 1986). It was found that the β-adrenergic receptor kinase does not phosphorylate such common substrates as mixed histones or casein. Moreover the β-adrenergic receptor kinase is not stimulated by common kinase activators such as cAMP, cGMP, Ca^{2+}/calmodulin or Ca^{2+}/phosphatidylserine indicating that the β-adrenergic receptor kinase is distinct from other known kinases (Benovic et al., 1986).

The homologous nature of desensitization is characterized by a selective blunting of the response to the desensitizing agonist. Thus, phosphorylation of the agonist-occupied form of the β-adrenergic receptor by βARK provides a mechanism which can account for the phenomenon of homologous desensitization. Our current understanding of the process of homologous desensitization can be outlined as follows. Initially the agonist binds to its receptor inducing a putative conformational change which enables the receptor to interact with the

guanine nucleotide regulatory protein N_s. This results in stimulation of adenylate cyclase. Independent of the generation of the second messenger cAMP the cytosolic receptor kinase becomes associated with the plasma membrane where it interacts with and phosphorylates the agonist-occupied form of the receptor. The phosphorylated receptors are uncoupled from their interaction with N_s (unpublished observations). The phosphorylated receptors are then sequestered away from the plasma membrane into a vesicular compartment (Harden, 1983; Sibley and Lefkowitz, 1985). Whether receptor phosphorylation represents the trigger for sequestration or whether this sequestered compartment represents a specific site for receptor dephosphorylation are questions requiring further investigation (Sibley et al., 1986).

The most remarkable property of βARK is its exquisite specificity for the agonist-occupied form of the β-adrenergic receptor. This situation is strikingly similar to the light adaptation process in the rod outer segment of the eye where rhodopsin phosphorylation is catalyzed by a specific rhodopsin kinase which phosphorylates only bleached rhodopsin (i.e. the "agonist" occupied form of the light receptor) (Bownds et al., 1972; Kuhn and Dreyer, 1972; Shichi et al., 1974, 1978). Rhodopsin phosphorylation attenuates the ability of rhodopsin to activate transducin, the nucleotide binding protein involved in this system (Shichi et al. 1984; Wilden et al., 1986). Thus, in addition to the similarities that exist in the functional components of these disparate systems (hormonal transduction and light perception) the discovery of a hormone receptor specific kinase suggests that these systems may share common regulatory mechanisms.

This homology has been further strengthened by the recent cloning of the gene for the hamster β-adrenergic receptor (Dixon et al., 1986). The β-adrenergic receptor and rhodopsin share several similar features including two glycosylation sites near the amino-terminus, seven putative trans-membrane helices, some amino acid homology and potential sites of phosphorylation. Phosphorylation of rhodopsin by rhodopsin kinase is known to occur primarily at serine and threonine residues clustered at the C-terminal 15 amino acids. The hamster β-adrenergic receptor also possesses a serine and threonine rich region in the last C-terminal 21 amino acids which may represent the site of βARK phosphorylation.

The S49 lymphoma cell, in particular the kin^- mutant which lacks protein kinase A, has served as a useful tool in the identification of a novel protein kinase (βARK) specific for the agonist occupied form of adenylate cyclase coupled receptors. This kinase may play an important

role in the process of homologous desensitization of adenylate cyclase reponsiveness. Moreover, the discovery of this enzyme greatly strengthens the homology which exists between such disparate systems as light transduction and hormone responsiveness.

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295P EFFECTS OF (+) AND RACEMIC SALBUTAMOL ON AIRWAY RESPONSES IN THE GUINEA-PIG

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In recent years, the incidence and severity of asthma, as well as associated death rates, have increased in several countries. It is appropriate therefore to ascertain whether anti-asthma drugs exhibit adverse effects that might contribute to these changes. An association between usage of beta-adrenoceptor agonist drugs and airway hyperreactivity in clinical asthma (Anonymous, 1990) has prompted study of (±)salbutamol, the most commonly used bronchodilator.

In the anaesthetised ventilated guinea-pig (Sanjar et al., 1990), reactivity of the airways to intravenous histamine (1.0-3.2 µg/kg) was enhanced significantly ($p < 0.01$, n=10,) following an intravenous infusion for one hour of (+)salbutamol (100 µg/kg), the non-bronchodilator enantiomer of racemic salbutamol. In studies with racemic salbutamol the bronchodilator action of (-) salbutamol precluded demonstration of airway hyperreactivity; hence, airway hyperreactivity was not detected following infusion of (±)salbutamol over 1 hour (100 µg/kg, n=10). However, increased responsivity to histamine was demonstrable four days after sustained subcutaneous infusion of (±)salbutamol (1 mg/kg/day, n=10), implying that the effect of (+)salbutamol on airway responsivity was less prone to tachyphylaxis than the spasmolytic effect of (-)salbutamol.

Subcutaneous infusion of (±)salbutamol (1 mg/kg) for more than two days increased the susceptibility of sensitised guinea-pigs to inhaled ovalbumin and caused almost 100 % mortality; an effect which was abrogated by inhalation of aerosolised (±)isoprenaline (0.1 % w/v) or subcutaneous injection of (±)salbutamol (1 mg/kg), immediately prior to inhalation of ovalbumin. Following subcutaneous infusion of (±)salbutamol (1 mg/kg, n=10) for 5 days, increased obstruction of the airways during inhalation or intravenous injection of ovalbumin was evident, which could account for death in such animals. Whether an increased incidence of neutrophils in the airway lumen observed 24 hours after inhalation of salbutamol (Boubekeur et al., 1989) contributed to the observed increase in airway reactivity has yet to be determined.

The capacity of (±)isoprenaline to induce airway hyperreactivity has been reported previously (Sanjar et al., 1990) and provides a plausible mechanism to account for the epidemic of asthma deaths twenty years ago (Speizer et al., 1968). In light of contemporary clinical evidence that bronchodilator therapy can be associated with enhanced airway reactivity, the pharmacology of (+)salbutamol and other (+)isomers of substituted catecholamines merits clinical investigation.

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296P NITRIC OXIDE AND ACETYLCHOLINE HYPERPOLARIZE SMOOTH MUSCLE CELLS IN THE RAT SMALL MESENTERIC ARTERY BY DIFFERENT MECHANISMS

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Acetylcholine and related cholinomimetics stimulate endothelium-dependent hyperpolarization and relaxation in arterial smooth muscle cells (Bolton et al, 1984; Taylor & Weston, 1988; McPherson & Angus, 1991). The differential sensitivity of the hyperpolarization and relaxation to various blocking agents has led to the suggestion that these events are mediated by separate endothelium-derived factors (Taylor & Weston, 1988). Recently, Tare & co-workers (1990) have demonstrated that nitric oxide, which appears to be or is closely related to EDRF, can stimulate smooth muscle hyperpolarization as well as relaxation, implying a role for nitric oxide in the endothelium-dependent hyperpolarization to acetylcholine. The present study investigated and compared the responses to both acetylcholine and nitric oxide in the rat mesenteric artery in a myograph.

Smooth muscle cells in isolated segments of rat small mesenteric artery had a resting potential around -57mV. Both acetylcholine and nitric oxide stimulated concentration-dependent hyperpolarization. The hyperpolarization to acetylcholine was endothelium-dependent, and increased the membrane potential to around -67mV. If the artery was first exposed to noradrenaline (1-3µM), the smooth muscle cells contracted, and were depolarized to -35mV. Acetylcholine again hyperpolarized the membrane to around -67mV with the highest concentration tested (3µM) and in addition, reversed the contraction by over 90%. Both the hyperpolarization and the relaxation were unaffected by the presence of glibenclamide (3µM). Nitric oxide (0.1-1µmole), applied either as a gas in solution or released from acidified sodium nitrite, produced a transient hyperpolarization of the resting membrane potential which varied between 3 and 9mV. Unlike acetylcholine, the hyperpolarization was abolished by prior smooth muscle depolarization in the presence of noradrenaline, although at this time nitric oxide stimulated marked smooth muscle relaxation. Glibenclamide (3µM) reversably blocked the hyperpolarization of the resting membrane potential which occurred in response to nitric oxide.

These data show that the smooth muscle hyperpolarizations to acetylcholine and nitric oxide are induced in different ways. The voltage-dependent block of hyperpolarization to nitric oxide suggests the involvement of inwardly-rectifying potassium channels, which because of their sensitivity to glibenclamide may be ATP-dependent.

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EXHIBIT 2

TF Albuterol J

Racemic mixtures at root of worsening symptoms?

Active enantiomers may cause adverse effects in asthma

In a recent discussion in *TiPS*[1], of mechanisms whereby β_2-adrenoceptor-selective sympathomimetic drugs might worsen asthma symptoms, Barnes and Chung make no mention of the possibility that enantiomers of these racemic mixtures might be culpable. Isoprenaline, salbutamol, salmeterol and terbutaline have one chiral centre and are racemic mixtures of two enantiomers, with β_2-adrenoceptor agonist activity residing in the R-enantiomers. Fenoterol and formoterol have two chiral centres, giving rise to two possible diastereomers each having two enantiomers and, although marketed as single diastereomers, they are racemic mixtures of the R,R- and S,S-enantiomers.

Although it is generally accepted that the activity of a single enantiomer accounts for the biological effects of sympathomimetics, potent biological properties, unrelated to adrenoceptor occupancy, are documented. For instance, racemic tretoquinol not only relaxes airway smooth muscle but is also a potent inhibitor of platelet activation. Relaxation of guinea-pig trachea can be attributed to the (−)-S-enantiomer ($pD_2 = 7.10$) rather than the (+)-R-enantiomer ($pD_2 = 5.54$)[2], whereas inhibition of human platelet aggregation by the thromboxane A_2 mimetic U46619 is a property of (+)-R-tretoquinol ($IC_{50} = 0.99 \pm 0.02$ μM) rather than (−)-S-tetroquinol ($IC_{50} = 39.6 \pm 4.3$ μM)[2].

The capacity of sympathomimetics to facilitate sudden death in response to inhaled allergen or airway spasmogens in the guinea-pig is long established[4]. In studying the mechanism whereby salbutamol increases susceptibility of the sensitized guinea-pig to airway spasmogens[5], we noted that intravenous infusion of (+)-S-salbutamol induces airway hyperreactivity to leukotriene C_4 (Ref. 6) by a mechanism closely analogous to that detailed for (+)-S-isoprenaline (i.e. unaffected by racemic propranolol but prevented by vagal section)[7].

More recently, we have observed that intratracheal instillation of S-isoprenaline, S-salbutamol and S-terbutaline are similarly efficacious in evoking increased airway responsivity to intravenous injection of histamine in the anaesthetized guinea-pig. Such observations demonstrate that enantiomers of sympathomimetics are not inert and hence may contribute to adverse effects of the type discussed by Barnes and Chung. It has long been recognized that use of sympathomimetics for asthma therapy is associated with a range of inconsistent, or frankly paradoxical, effects[8]. Rather than adding further material (i.e. glucocorticosteroids) to existing products as proposed, our findings indicate that it may be prudent to remove enantiomers that were previously thought to be biologically inert.

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U46619: 9,11-dideoxy-11α-epoxymethano-prostaglandin $F_{2\alpha}$

TIPS 13 231-232 (1992)

EXHIBIT 3